Amylin/CGRP

Lutz, T A

Abstract: This chapter focuses on the anorectic action of the pancreatic hormone amylin and the structurally related peptide calcitonin-gene related peptide (CGRP). Both peptides when given peripherally or centrally produce potent anorectic effects and delay gastric emptying. The anorectic action of CGRP seems to parallel amylin's effect on eating in many aspects; however, in this chapter, more emphasis will be put on the effects of amylin on eating because amylin's effects may be of physiological relevance but this is less clear in the case of CGRP. Other effects of CGRP are mentioned in other chapters of this book.

DOI: https://doi.org/10.1016/B978-0-12-385095-9.00140-8

Other titles: Role of amylin and calcitonin-gene related peptide (CGRP) in the control of eating (ursprünglicher Titel der geändert wurde in Amylin/CGRP)

Role of amylin and calcitonin-gene related peptide (CGRP) in the control of eating

Thomas A. Lutz

Institute of Veterinary Physiology

Vetsuisse Faculty University of Zurich

Winterthurerstrasse 260

8057 Zurich

phone +41-44-635 8808

fax +41-44-635 8932

e-mail tomlutz@vetphys.unizh.ch

Abstract

This chapter focuses on the anorectic action of the pancreatic hormone amylin and the structurally related peptide calcitonin-gene related peptide (CGRP). Both peptides when given peripherally or centrally produce potent anorectic effects and delay gastric emptying. The anorectic action of CGRP seems to parallel amylin's effect on eating in many aspects; however, in this chapter, more emphasis will be put on the effects of amylin on eating because amylin's effects may be of physiological relevance but this is less clear in the case of CGRP. Other effects of CGRP are mentioned in other chapters of this book.

Key words

Satiation; adiposity; amylin interactions; anti-obesity therapy.

Introduction

The acute and chronic eating inhibitory effects of amylin seem to be one important factor in amylin's overall role to control the influx of nutrients into the circulation. Apart from this effect, amylin's action to reduce gastric acid secretion, to limit the rate of gastric emptying, and to diminish pancreatic glucagon and digestive enzyme secretion are other factors serving the same purpose (12, 29). Therefore, by regulating nutrient appearance and the postprandial glucose concentration, amylin seems to be a necessary and complementary factor to insulin in the control of nutrient flux. Amylin, or islet amyloid polypeptide (IAPP), was first isolated from pancreatic amyloid deposits, which typically occur in human type 2 diabetes mellitus, in feline diabetes mellitus, and in insulinomas in animals and humans. Amylin, however, is also a physiological product of pancreatic B-cells from where it is co-secreted with insulin. It is generally assumed that the pancreatic B-cells constitute the main source for circulating and postprandially released amylin, and that the increase in amylin levels in response to eating is the physiological basis for amylin's satiating effect. Accordingly, pancreatectomized cats have very low baseline amylin (and insulin) levels and virtually no postprandial increase in blood amylin levels (unpublished); unfortunately, no measures of eating or meal pattern were determined in these studies.

Eating results in a marked increase in the plasma amylin concentration. This meal-induced increase is rapid and correlates with the size of the pertinent meal. Blood levels of CGRP show only a modest and late increase in response to eating. Therefore, a physiological role for CGRP in satiation appears questionable, at least referring to a hormonal action of circulating CGRP. The source for this increase in circulating CGRP may be by spillover from neuronal cells.

Receptors for amylin and CGRP action (9, 13, 24)

Amylin and CGRP binding sites belong to the family of G-protein coupled receptors. The functional amylin receptor involves the calcitonin receptor (CT-R) as a core receptor, whose amylin-specificity and affinity is due to the co-expression of one of several receptor activity

modifying proteins (RAMPs). The CT-R without co-expression of RAMPs represents the classical CT receptor, whereas a typical amylin receptor arises from the interaction of RAMP 1 or RAMP 3 with the CT-R. A similar principle leads to functional receptors for CGRP; they rely on the co-expression of the calcitonin receptor-like receptor (CL-R) with appropriate RAMPs.

Amylin binding sites are widely distributed in the central nervous system and also occur in high densities in brain areas involved in the control of eating. The area postrema (AP) which seems to mediate amylin's and CGRP's effects on eating, contains all components of functional amylin receptors, as shown by the presence of RAMP1 and RAMP3 mRNA and positive immunostaining for RAMP1, RAMP3 and the CT-R.

Detailed studies of the presence of CL-R protein as part of functional CGRP receptors in the brain, and especially in brain areas involved in the control of eating are not available. However, in situ hybridization studies have shown that CL-R mRNA is present in the rat brain. Other than in the AP, the physiological relevance of amylin and CGRP binding sites for the anorectic actions of amylin and CGRP remains unknown.

Characterization of the effects of amylin and CGRP on eating as satiating hormone (12)

Amylin shares the typical characteristics of satiating hormones which are involved in the control of meal size. Amylin is rapidly released during food ingestion, and it dose-dependently reduces meal size. Amylin has a rapid onset and brief duration of action, and amylin antagonism increases eating by a specific meal size effect (14, 20).

In contrast to some other satiating hormones like cholecystokinin (CCK), amylin is also active under conditions of chronic delivery; amylin reduced eating by decreasing average meal size but it may also reduce meal number by increasing the duration of the intermeal interval. Similarly, amylin increased the latency to eat under certain experimental conditions. Nonetheless, the best characterized function of amylin is the effect to reduce meal size.

Qualitatively, CGRP reduces eating by affecting the same meal pattern parameters as amylin; i.e., CGRP has also been shown to reduce meal size after acute and chronic administration, and to affect the intermeal interval during chronic infusion. Due to the lack of 100% specific antagonists against amylin or CGRP receptors and the lack of studies using specific receptor deficient animals, it remains unclear whether CGRP's effects on eating are due to an interaction at amylin receptors or due to a specific, amylin independent effect. However, based on its release pattern with an increase of plasma CGRP levels only occurring at least 1h after meal onset, it appears unlikely that blood borne CGRP constitutes a physiological satiating agent (7).

Meal pattern analysis after the injection of amylin or CGRP revealed another potential difference between amylin and CGRP action because CGRP reduced the eating rate in rats, at least when using higher doses. This suggests that some aversive or illness-producing effect may at least partly underlie the anorectic action of CGRP, indicating that its action on feeding is not specific. This contrasts with amylin which does not reduce feeding by producing a conditioned taste aversion or by an unspecific effect (e.g. via a reduction in drinking) (12).

The lowest dose of exogenous amylin that produced a significant reduction in feeding yielded plasma amylin levels that were about two-times higher than the concentrations measured postprandially. Strictly speaking, this means that physiological amylin concentrations that occur at the end of spontaneous meals have not yet been shown to reduce feeding. However, different kinetics of the blood amylin concentration after exogenous amylin delivery rather than endogenous secretion may play a role. Further, under natural feeding conditions many gastrointestinal hormones that individually induce satiation are released at the same time and may interact in a way that may be difficult to mimic in an experimental setting. Overall, we believe that amylin is a physiological control of meal size and that this effect is based on circulating amylin; importantly, several studies have clearly shown that peripherally or centrally delivered amylin antagonists produce an effect opposite to that of amylin, i.e., an increase in meal size (e.g, (14)).

Amylin as an adiposity signal

The role of amylin as a satiating hormone seems to depend on phasic changes in amylin levels, i.e. the meal-induced rise in plasma amylin levels. In addition, amylin may also exert a tonic effect on eating, i.e. it may play a role in the long-term control of eating or body weight (10, 27).

First, basal levels of amylin also seem to depend on the prevailing body weight and body adiposity, in both rats and humans. High-fat fed, obese rats have higher baseline amylin levels than age matched lean controls; of note, this does not distinguish between a body weight and a diet effect. We recently also investigated whether changes in body adiposity in single individuals are reflected in changing amylin levels and whether such changes in adiposity and amylin in fact follow the same temporal pattern; rats were rendered overweight by chronic intragastric overfeeding leading to a 30% excess body weight above normal weight rats. To our surprise, basal amylin levels did not change significantly during or after termination of overfeeding. Whether amylin levels may only increase after extended periods of overweight or whether the type of diet used during overfeeding may play a role is currently unknown. It seems clear that further studies are required to define the exact contribution of time, diet, extent of overweight and other potential factors on the obesity related increase in plasma amylin levels.

Second, chronic amylin infusion in rats lowers eating and body weight gain by reducing body adiposity while chronic peripheral or central infusion of amylin antagonists increases body weight with a major effect on body fat mass while sparing lean body mass (20, 23). We recently showed that chronic third ventricular amylin infusions reduce body weight gain in rats irrespective of prior weight manipulations. This means that amylin infused rats, whether temporarily food restricted, overfed or left unmanipulated, eventually reached similar body weight which was consistently lower than in saline infused controls (27). Hence, the effect of a chronic modulation of amylin signaling is similar to that of leptin or insulin. The relative contribution of amylin versus leptin or insulin to the control of body weight is difficult to judge but it is clear that the lack of leptin in *ob/ob* mice results in a more dramatic obese phenotype than the lack of amylin in amylin deficient mice (8, 12). However, because obesity associated leptin resistance prevents leptin from being

effective when the effect apparently would be needed most, additional amylin may be a very effective alternative and amylin may play an important modulatory role. Further, even animals with defective leptin and insulin signaling (e.g. the Zucker *fa/fa* rat) keep their body weight relatively stable, even though this occurs at a higher level. Hence, amylin may play some role as a adiposity feedback signal similar to leptin and insulin, at least when the leptin and insulin feedback signalling systems are deficient.

Eating in knockout animals (16)

Amylin knockout animals have been developed to study the role of amylin in nutrient and bone metabolism (8). The specific phenotypes of amylin-deficient mice that are not related to amylin's role in the control of eating include a lower bone mass due to a higher number of osteoclasts, and reduced late phase nociception in the paw formalin test; the latter implies that endogenous amylin increases the perception of chemical pain.

In the same animals, adult body weight did not differ between *knockout* and *wildtype* animals in some studies, but amylin *knockout* mice showed a slightly higher rate of body weight gain compared with corresponding *wildtype* controls; the *knockout* mice grew significantly faster up to about 4 months of age. Eating was slightly though not significantly higher than in control animals (12).

Interestingly, amylin *knockout* mice also differed from control animals in their interaction with other factors controlling eating, indicating that endogenous amylin may have a facilitating role for other controls of eating (e.g., CCK (15); or leptin). Together, these studies suggest a role for endogenous amylin in the overall control of energy balance, most likely via an effect on eating and in interaction with other satiating and adiposity signals (12).

Eating in amylin receptor *knockout* animals has not been determined so far. A recent study determined the effect of haplodeletion of amylin and its receptor protein (calcitonin receptor; CT-R)

on bone metabolism. Heterozygous amylin$^{+/-}$ and heterozygous CT-R$^{+/-}$ mice were crossbred, but neither data on eating nor on body weight were reported (16).

Eating in animals overexpressing amylin (16)

To my knowledge, no detailed studies are available on the effect of amylin overexpression on eating or body weight. Rats that were transgenic for the overexpression of human amylin gained weight comparably until 5 months of age but then had a slightly lower body weight than wild-type controls (4). The authors argued that lower body weight may have been a consequence of energy loss due to the development of glycosuria and diabetes in the transgenic rats, but no detailed data on eating were provided. Hence, reduced eating which in principle would be consistent with the effects of exogenous amylin on eating and body weight, cannot be excluded. The rather small difference between transgenic and control animals may have been due to receptor desensitization or the redundant system controlling body weight. Because the major difference in body weight between amylin *knockout* and *wildtype* mice occurs during a certain period of life, the same may also hold true for animals overexpressing amylin. Another recent paper with mice that were transgenic for the overexpression of human amylin did not report data on eating and body weight (28).

Eating in animals deficient of CGRP or overexpressing CGRP (16)

While no detailed data are available related to the effect of CGRP overexpression on eating or body weight, a recent paper described the effect of genetic CGRP deficiency (CGRP ko) on eating and body weight in mice. CGRP ko mice fed different diets ate slightly but significantly more than control mice of the same diet group; total eating and eating corrected for body weight gain were both increased significantly. This effect on eating in principle is consistent with exogenous CGRP's effect to reduce energy intake. Surprisingly, however and despite overeating, the ko mice appeared to be protected from the development of diet-induced obesity because both their body weight and body adiposity were lower than in wildtype animals. The latter effect may be explained by the increase in core body temperature and energy expenditure in ko versus wildtype mice (28).

Energy expenditure as affected by amylin and CGRP (reviewed in: (12); see also: (27))

A number of studies have shown that acute (central) and chronic amylin administration increase energy expenditure as assessed by indirect calorimetry. The effect under chronic conditions may in part be secondary to the reduction in adiposity by amylin and hence the relative increase in metabolically more active lean body mass. Acute peripheral injection of an anorectic dose of amylin failed to increase energy expenditure in rats while the long acting amylin agonist salmon calcitonin (sCT) increased it; we therefore believe that this difference may be due to the different biological half life of amylin versus sCT. The central neural targets which may mediate the effect of exogenous amylin on energy expenditure and the underlying mechanisms are not clear.

Given the similar eating inhibitory effects of amylin and CGRP, the recent report using CGRP ko mice that indicated that the lack of CGRP resulted in increased rather than decreased energy expenditure was surprising. Whether CGRP replacement would have corrected for this phenotype was not tested (28).

Energy metabolism in animals with increased amylin or CGRP signaling propensity (30)

A recent study provided interesting insight into the potential mechanisms of enhanced amylin or CGRP receptor signaling. This study used transgenic mice overexpressing the human RAMP1 gene specifically in the central nervous system; because RAMP1 is a chaperone to the amylin (CT-R) and CGRP (CL-R) receptors, any effect observed in these mice could be due to enhanced signaling of amylin, of CGRP or both. Total eating was reduced temporarily in the transgenic mice, and eating related to body weight gain was rather increased. Interestingly, energy expenditure was significantly higher in the transgenic mice, probably explaining the lower body weight and adiposity. Because the effect of exogenous central amylin but not CGRP on body weight gain was markedly enhanced in the transgenic mice, the phenotype of the RAMP1 transgenic mice may be mainly due to enhanced brain amylin signaling via the CT-R/RAMP1 receptor complex. This may

also explain the apparent paradox between lower body adiposity in these mice, and the effect of the knockout of CGRP in mice that resulted in a protection from diet-induced obesity (28).

Regulation of amylin and CGRP synthesis and secretion in lean and obese individuals (2, 3)

Due to their co-synthesis and co-localization in secretory vesicles, insulin and amylin are normally co-secreted upon activation with the appropriate stimuli; this typically occurs at a fixed ratio of about 1:10 to 1:100. Interestingly, divergent secretion of insulin and amylin is possible but it remains unclear how differential release of insulin and amylin is regulated. Human obesity, various rat models of obesity, diabetes mellitus, pancreatic cancer, and pharmacological intervention (e.g. dexamethasone) may lead to relative over-expression and -secretion of amylin versus insulin. Clear evidence for a preferential transcription of amylin or increased stability of amylin mRNA under these conditions has not been found so far. It has however been hypothesized that differential regulation of amylin and insulin mRNA production may involve the cAMP and protein kinase A (PKA) pathways, and that different transcription factors for insulin and amylin may be involved.

We recently investigated if obesity or maintenance on a high fat diet (HF diet) affect meal induced amylin release in rats. HF fed rats, whether classified as diet-induced obese (DIO) or diet-resistant (DR) exhibited an earlier meal-induced rise in amylin compared to lean chow-fed controls. Further, DR rats had higher amylin:insulin ratios before and after a meal. In principle, these findings are consistent with the demonstration that elevated fatty acids, which can result from the consumption of a diet high in fat, induce enhanced mRNA expression and release of amylin, but not insulin. One important overall conclusion from these experiments in respect to amylin's role in the control of eating and in obesity was that amylin secretion does not seem to be blunted in obesity; it therefore seems unlikely that insufficient release of amylin contributes to overeating in obesity or in individuals on HF diets.

Obese individuals have been reported to have higher CGRP levels than lean controls (7), but the physiological relevance of this finding is unclear. A direct link between baseline CGRP levels and the degree of adiposity appeared unlikely because CGRP did not decrease in response to body weight loss. Plasma levels of CGRP increase in response to eating mainly by spillover from sensory nerve endings rather than controlled release from endocrine cells. This was only seen after a highfat, but not after a high carbohydrate meal, and the late increase (> 1h) argues against an effect of CGRP on individual meals. Specific studies directly comparing the meal induced changes in CGRP secretion and blood concentrations in lean versus obese individuals or in individuals on specific diets have not been reported.

Central pathways mediating the anorectic effects of amylin and CGRP (reviewed in: (12, 17))

Experimental evidence supports the idea that the anorectic effect of peripheral amylin is mediated by direct humoral action on the area postrema (AP) in the hindbrain. Amylin binding sites have been described in various CNS locations involved in the control of eating, but behavioral tests with peripheral or local AP infusions, immunohistological and electrophysiological experiments all indicate that the AP seems to be necessary as primary site of action for peripheral amylin to reduce eating.

Blood borne amylin has easy access to AP neurons due to the lack of a functional blood brain barrier in this area; several studies from different laboratories have shown that peripheral amylin's anorectic action is abolished in rats with lesions in the AP. Endogenous blood borne amylin also appears to act via the AP because an infusion of the amylin antagonist AC 187 into the AP increased eating due to an increase in meal size; further, AC 187 infused into the AP also partly blocked the anorectic effect induced by a peripheral amylin injection, providing the best in vivo evidence for the importance of the AP in mediating peripheral amylin's anorectic action. Electrophysiological and immunohistochemical studies indicate that AP neurons are directly and dose-dependently stimulated by amylin, and that exogenous and endogenous amylin produced a

strong expression of the immediate early gene product c-Fos protein as a marker of neuronal activation in AP neurons. Both responses were effectively blocked by AC 187.

Phenotype of amylin responsive neurons in the AP (19)

Expression of c-Fos protein was used to determine the phenotype of amylin responsive neurons in the AP. We found that about 50% of amylin-activated AP neurons are noradrenergic because they expressed dopamine-beta-hydroxylase (DBH), the enzyme catalyzing noradrenaline (NA) synthesis. These neurons seem to play a specific functional role in amylin's effect on eating because even a partial (approx. 50%) lesion of the NA-containing neurons in the AP was sufficient to block the acute eating effect of peripheral amylin. Further, amylin did not induce a significant c-Fos response in AP neurons in NA-lesioned rats.

Intracellular signaling pathways in AP neurons activated by amylin (12, 17)

We believe that the amylin induced c-Fos response in the AP has no functional implications because the kinetics of c-Fos expression and the onset of amylin's anorectic effect are inconsistent. We recently provided evidence for a functional role of phosphorylation of the extracellular signal regulated kinase (pERK1/2) cascade in amylin's effect; amylin induced ERK1/2 phosphorylation, and (partial) blockade of this effect attenuated amylin's feeding response. How this relates functionally to amylin induced cGMP formation in AP neurons remains to be investigated.

Projections of AP neurons implicated in amylin's and CGRP's effects and hypothalamic involvement in the anorectic action of peripheral amylin (18, 21)

Amylin elicits a strong c-Fos response in AP projection areas, namely the nucleus of the solitary tract (NTS), the lateral parabrachial nucleus (lPBN), the central nucleus of the amygdala (CeNA), and the bed nucleus of the stria terminalis (BNST). The AP, NTS, and the lPBN form a necessary part of the CNS pathway conveying amylin's anorectic signal to higher brain structures; their activation seems to be secondary to an action of amylin on AP neurons.

Retrograde and anterograde tracer studies confirmed reciprocal projections between these amylin activated areas. We identified dense projections from the amylin activated neurons in the lPBN and sparse projections from the NTS to the dorsal lateral hypothalamus (dLHA) where amylin reduces fasting induced c-Fos expression; hence, the lPBN may thus mediate the amylin-induced inhibition of the dLHA. This inhibition may be linked to the down-regulation of the expression of orexin and melanin-concentrating hormone in the dLHA.

Dense efferents were also observed from the lPBN to other hypothalamic areas, namely to the ventromedial (VMH), dorsomedial, paraventricular (PVN) and arcuate nuclei (ARC). Their functional role is not entire clear, but see paragraphs on amylin/leptin interactions and the role of the ventromedial hypothalamus in this respect.

Site of CGRP action

The site of action for CGRP's anorectic action has been investigated in less detail than that of amylin. Similar to amylin, the anorectic action of peripheral CGRP is abolished in rats with an AP lesion. Immunohistochemical and electrophysiological studies have not been performed to the same extent as for amylin, so that it is difficult to integrate these findings into the whole brain network for the physiological control of eating.

Forebrain effects of amylin (12)

The role of forebrain amylin receptors is still unclear. It seems likely that these receptors mediate the anorectic action of amylin infused into the third brain ventricle; this effect is very potent and long-lasting, and third ventricular infusion of AC 187 increases eating in rats. The latter is associated with an increase in body weight and the weight of the retroperitoneal fat pads. Nonetheless, the functional relevance of these findings under physiological conditions is not clear and at present there is no evidence to suggest that forebrain amylin receptors are involved in the regulation of eating by peripheral amylin.

CNS production of amylin has not been shown unequivocally, except in a recent interesting study pointing to a potential role of hypothalamic amylin in the modulation of maternal behavior (6). The physiological relevance of these findings is currently under investigation.

Forebrain effects of CGRP

Injection of CGRP into the PVN which contains CGRP binding sites (5) produced a strong anorectic effect, and it increased the concentration of various anorectic neuropeptides in the hypothalamus. However, the physiological relevance of these findings is unclear because very high doses of CGRP had been used, exceeding the EC_{50} for amylin to elicit an anorectic effect after administration into the third brain ventricle by a factor of more than 200. Further, the PVN content of CGRP was elevated in response to fasting, which is difficult to reconcile with the idea of CGRP acting as an anorectic hormone or neuropeptide. Hence, the role of CGRP as a brain-intrinsic neuropeptide in the control of feeding remains to be investigated.

Interaction between amylin and estradiol (11, 25)

Only few studies tested the specific anorectic effect of amylin in females. Similar to CCK and other satiating hormones, single acute injections of amylin were more effective in ovariectomized rats with physiological estradiol replacement than in rats without replacement. Further, the amylin antagonist AC187 increased eating more in estradiol replaced ovariectomized animals, pointing to the physiological relevance of the modulating effect of estradiol on amylin action. Surprisingly, the effect was opposite under chronic conditions; in other words, the action of chronic amylin on eating and body weight was stronger in ovariectomized rats that lacked estradiol than in control rats or in ovariectomized rats receiving estradiol replacement. Chronic amylin also at least partly restored the metabolic rate that was decreased as a consequence of ovariectomy. Hence, the interaction between amylin and estradiol seems to be complex and these apparently opposing phenomena require intensified studies.

Short-term interaction with other hormones controlling ingestive behavior (12, 22)

Subthreshold doses of peripheral insulin and amylin reduce eating in rats when co-administered, and central insulin enhanced the action of peripheral amylin. Further, amylin and CCK also seem to interact in their effect on eating. CCK and amylin synergistically reduced eating; similar synergistic effects have been reported with PYY and for the combination of sCT with the GLP-1 agonist exendin-4 in non-human primates (1). Further, part of the satiating action of CCK seems to be mediated by amylin, because amylin antagonists attenuated CCK's anorectic actions in rats and because the anorectic effect of CCK was almost abolished in amylin *knockout* mice; the latter effect was restored by amylin. In other words, endogenous amylin seems to modulate CCK's anorectic effect.

Interaction between amylin and leptin (22, 26)

Amylin and leptin seem to interact acutely and chronically. Acute central leptin increased the eating-inhibitory effect of peripheral amylin. Further, two-week peripheral infusions of amylin and leptin were performed in leptin resistant DIO rats. Amylin alone reduced eating and led to a decrease in body weight. Interestingly, rats pair fed to the amylin group and that received leptin did not lose more weight than amylin-treated rats. However, combined application of exogenous leptin and amylin decreased eating and body weight more than amylin alone, and body fat was lowest after leptin/amylin. Hence, amylin strongly enhanced the sensitivity of obese rats to the catabolic effect of leptin, including an increase in energy expenditure. Body fat loss was more in the amylin/leptin treated rats than in the pair-fed controls; this was consistent with a lower respiratory quotient, indicating preferential fat oxidation. One important point to consider is that the interactions of amylin and leptin on eating and adiposity have mainly been shown under pharmacological, not under physiological conditions.

Role of the VMH in the leptin and amylin interaction (26)

The interaction between amylin and leptin appears to be due to a direct effect of amylin on central leptin signalling, most likely in the VMH and potentially the hypothalamic ARC. Amylin enhanced leptin signalling as gauged by increased immunoreactivity of pSTAT3 specifically in the VMH; this effect may be linked to an amylin-induced upregulation of leptin receptor expression and hence direct enhancement of leptin action in VMH neurons. Further, amylin deficient mice had a reduced leptin receptor expression in the mediobasal hypothalamus.

Interaction between amylin and metabolites (12)

Higher doses of amylin are necessary to inhibit sham feeding than real feeding which may be due to the lack of cooperative effects between amylin and metabolites (e.g., elevated glucose) under these conditions. Similar to amylin's effect on gastric emptying, amylin's eating inhibitory effect seems to be reduced in rats with induced hypoglycemia. This is in line with coactivation of AP neurons by physiological concentrations of amylin and glucose. The co-responsiveness of AP neurons to amylin and glucose is similar to that for glucose and CCK; AP neurons may therefore integrate metabolic and hormonal signals that control eating.

Physiological and pathophysiological implications

Relatively little is known about the possible role of amylin's effect on feeding under pathophysiological conditions. It has been hypothesized that amylin may contribute to cancer anorexia in certain pancreatic neoplasia which may be associated with chronically supraphysiological plasma amylin levels.

An altered ratio between amylin and insulin has been supposed to play a role in eating disorders and hence to contribute to abnormal feeding behavior in diabetes mellitus. In other words, the lack of amylin in type 1 diabetes and later stages of type 2 diabetes could result in increased eating or body weight. The clinical experience with the amylin analogue pramlintide in the treatment of type 1 and 2 diabetes is consistent with this idea. Individuals treated with pramlintide plus insulin lost body weight while patients treated with insulin alone gained weight. Subsequent trials showed that

pramlintide decreased in eating and body weight in obese or diabetic patients. Because obesity and chronic hyperamylinemia, which is typically associated with obesity, do not seem to reduce amylin effectiveness, amylin may be a promising and potent approach to treat obese patients. Similar to the data in rats, this effect can be potentiated when amylin is combined with leptin.

Similar data are not available for CGRP. Hence, it is unknown whether CGRP or CGRP analogues produce similar or even more potent effects than amylin in a clinical setting. Given the current availability of data, amylin seems to be a more promising and safer approach than CGRP.

Summary

Both amylin and the structurally related neuropeptide CGRP reduce eating. While amylin may be a physiological control of meal size, this is less clear for CGRP. The effects of peripheral amylin and CGRP on eating are centrally mediated by the AP in the hindbrain as the most likely primary target area. Chronic exposure to amylin decreases eating and body weight gain. Next to a satiating action, amylin may also constitute an adiposity feedback signal. The anorectic action of amylin is pharmacologically exploited because amylin analogs are under clinical consideration for their effect to reduce eating and body weight in humans; this effect can be potentiated with leptin co-therapy.

Selected reading

1. **Bello NT, Kemm MH, Ofeldt EM, and Moran TH**. Dose combinations of exendin-4 and salmon calcitonin produce additive and synergistic reductions in food intake in nonhuman primates. *Am J Physiol Regul Integr Comp Physiol* 299: R945-952, 2010.

2. **Boyle CN, and Lutz TA**. Amylinergic control of food intake in lean and obese rodents. *Physiol Behav* 2011.

3. **Boyle CN, Rossier MM, and Lutz TA**. Influence of high-fat feeding, diet-induced obesity, and hyperamylinemia on the sensitivity to acute amylin. *Physiol Behav* 104: 20-28, 2011.

4. **Butler AE, Jang J, Gurlo T, Carty MD, Soeller WC, and Butler PC**. Diabetes due to a progressive defect in beta-cell mass in rats transgenic for human islet amyloid polypeptide (HIP Rat): a new model for type 2 diabetes. *Diabetes* 53: 1509-1516, 2004.

5. **Dhillo WS, Small CJ, Jethwa PH, Russell SH, Gardiner JV, Bewick GA, Seth A, Murphy KG, Ghatei MA, and Bloom SR**. Paraventricular nucleus administration of calcitonin gene-related peptide inhibits food intake and stimulates the hypothalamo-pituitary-adrenal axis. *Endocrinology* 144: 1420-1425, 2003.

6. **Dobolyi A**. Central amylin expression and its induction in rat dams. *J Neurochem* 111: 1490-1500, 2009.

7. **Geary N**. Effects of glucagon, insulin, amylin and CGRP on feeding. *Neuropeptides* 33: 400-405, 1999.

8. **Gebre-Medhin S, Mulder H, Pekny M, Westermark G, Tornell J, Westermark P, Sundler F, Ahren B, and Betsholtz C**. Increased insulin secretion and glucose tolerance in mice lacking islet amyloid polypeptide (amylin). *Biochem Biophys Res Commun* 250: 271-277, 1998.

9. **Hay DL, Christopoulos G, Christopoulos A, Poyner DR, and Sexton PM**. Pharmacological discrimination of calcitonin receptor: receptor activity-modifying protein complexes. *Mol Pharmacol* 67: 1655-1665, 2005.

10. **Isaksson B, Wang F, Permert J, Olsson M, Fruin B, Herrington MK, Enochsson L, Erlanson-Albertsson C, and Arnelo U**. Chronically administered islet amyloid polypeptide in rats serves as an adiposity inhibitor and regulates energy homeostasis. *Pancreatology* 5: 29-36, 2005.

11. **Lutz TA**. Amylin may offer (more) help to treat postmenopausal obesity. *Endocrinology* 152: 1-3, 2011.

12. **Lutz TA**. The role of amylin in the control of energy homeostasis. *Am J Physiol Regul Integr Comp Physiol* 298: R1475-1484, 2010.

13. **McLatchie LM, Fraser NJ, Main MJ, Wise A, Brown J, Thompson N, Solari R, Lee MG, and Foord SM**. RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. *Nature* 393: 333-339, 1998.

14. **Mollet A, Gilg S, Riediger T, and Lutz TA**. Infusion of the amylin antagonist AC 187 into the area postrema increases food intake in rats. *Physiol Behav* 81: 149-155, 2004.

15. **Mollet A, Meier S, Grabler V, Gilg S, Scharrer E, and Lutz TA**. Endogenous amylin contributes to the anorectic effects of cholecystokinin and bombesin. *Peptides* 24: 91-98, 2003.

16. **Muff R, Born W, Lutz TA, and Fischer JA**. Biological importance of the peptides of the calcitonin family as revealed by disruption and transfer of corresponding genes. *Peptides* 25: 2027-2038, 2004.

17. **Potes CS, and Lutz TA**. Brainstem mechanisms of amylin-induced anorexia. *Physiol Behav* 100: 511-518, 2010.

18. **Potes CS, Lutz TA, and Riediger T**. Identification of central projections from amylin-activated neurons to the lateral hypothalamus. *Brain Res* 1334: 31-44, 2010.

19. **Potes CS, Turek VF, Cole RL, Vu C, Roland BL, Roth JD, Riediger T, and Lutz TA**. Noradrenergic neurons of the area postrema mediate amylin's hypophagic action. *Am J Physiol Regul Integr Comp Physiol* 299: R623-631, 2010.

20. **Reidelberger RD, Haver AC, Arnelo U, Smith DD, Schaffert CS, and Permert J**. Amylin receptor blockade stimulates food intake in rats. *Am J Physiol Regul Integr Comp Physiol* 287: R568-574, 2004.

21. **Riediger T, Zuend D, Becskei C, and Lutz TA**. The anorectic hormone amylin contributes to feeding-related changes of neuronal activity in key structures of the gut-brain axis. *Am J Physiol Regul Integr Comp Physiol* 286: R114-122, 2004.

22. **Roth JD, Trevaskis JL, Turek VF, and Parkes DG**. "Weighing in" on synergy: preclinical research on neurohormonal anti-obesity combinations. *Brain Res* 1350: 86-94, 2010.

23. **Rushing PA, Hagan MM, Seeley RJ, Lutz TA, D'Alessio DA, Air EL, and Woods SC**. Inhibition of central amylin signaling increases food intake and body adiposity in rats. *Endocrinology* 142: 5035, 2001.

24. **Sexton PM, Paxinos G, Kenney MA, Wookey PJ, and Beaumont K**. In vitro autoradiographic localization of amylin binding sites in rat brain. *Neuroscience* 62: 553-567, 1994.

25. **Trevaskis JL, Turek VF, Wittmer C, Griffin PS, Wilson JK, Reynolds JM, Zhao Y, Mack CM, Parkes DG, and Roth JD**. Enhanced amylin-mediated body weight loss in estradiol-deficient diet-induced obese rats. *Endocrinology* 151: 5657-5668, 2010.

26. **Turek VF, Trevaskis JL, Levin BE, Dunn-Meynell AA, Irani B, Gu G, Wittmer C, Griffin PS, Vu C, Parkes DG, and Roth JD**. Mechanisms of amylin/leptin synergy in rodent models. *Endocrinology* 151: 143-152, 2010.

27. **Wielinga PY, Lowenstein C, Muff S, Munz M, Woods SC, and Lutz TA**. Central amylin acts as an adiposity signal to control body weight and energy expenditure. *Physiol Behav* 101: 45-52, 2010.

28. **Wong WP, Scott DW, Chuang CL, Zhang S, Liu H, Ferreira A, Saafi EL, Choong YS, and Cooper GJ**. Spontaneous diabetes in hemizygous human amylin transgenic mice that developed neither islet amyloid nor peripheral insulin resistance. *Diabetes* 57: 2737-2744, 2008.

29. **Young A, and Denaro M**. Roles of amylin in diabetes and in regulation of nutrient load. *Nutrition* 14: 524-527, 1998.

30. **Zhang Z, Liu X, Morgan DA, Kuburas A, Thedens DR, Russo AF, and Rahmouni K**. Neuronal Receptor Activity Modifying Protein-1 Promotes Energy Expenditure in Mice. *Diabetes* 2011.